

THE INFLUENCE OF THE ORIENTATION, ROUGHNESS, AND WETTABILITY OF SOLID SURFACES ON THE BEHAVIOR AND ATTACHMENT OF PLANULAE OF *CYANEA* (CNIDARIA: SCYPHOZOA)

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ABSTRACT

In the laboratory the planulae of *Cyanea* are exposed to a choice of substrates. Their behavior, the length of time required for them to attach, and the size they attain after attachment differs between glass (hydrophilic) and plastic (hydrophobic) surfaces. The correlation of these differences with wettability is shown by giving planulae a choice of six substrates exhibiting different degrees of wettability (determined by measurement of contact angles); planular attachment to these surfaces is directly proportional to the hydrophobicity of the substrate for those with contact angles from 17° to 82°, but their response is variable and relatively weak to the more hydrophilic surfaces. The same relationship between planular settlement and surface wettability is possibly shown by the preferred attachment of scyphozoan planulae (three species) to roughened plastic surfaces paralleling the increased hydrophobicity of this surface that roughening effects. However, a favorable surface (plastic) does not supercede the requirement that it also must be horizontally oriented to permit attachment to its lowermost surface; planulae rarely attach to uppermost surfaces, nor to vertically oriented surfaces.

Planulae exposed to natural substrate do not usually attach to clean shells in the laboratory nor are they found attached to fresh shells in the field, both of which have wettabilities equivalent to or less than that of glass. Aged shells, on the other hand, possessing organic and bacterial films, show increased hydrophobicity, and have a high incidence of planular attachment in the field.

INTRODUCTION

Free-living larvae characteristically attach to substrate suitable for the survival of subsequent benthic phases of their life history. This phenomenon is documented for numerous marine larvae (Meadows and Campbell, 1972; Crisp, 1974; Scheltema, 1974; Chia and Rice, 1978). Many of these investigations focus on the behavior of larvae in response to physical features of their environment (levels of illumination, temperature, salinity, current speed, substrate texture) or to specific biological substrates, such as algae on which the attached stage of a particular species is usually found; algal extracts applied as films to otherwise unfavorable surfaces induce these larvae to settle (*e.g.*, Williams, 1964; Nishihira, 1968; Ryland, 1976). Other organic films (Crisp and Meadows, 1963) and bacterial populations (Meadows and Williams, 1963; Müller, 1969; Cameron and Hinegardner, 1974; Brancato and Woollacott, 1982; Kirchman *et al.*, 1982a, b) influence the extent of larval attachment, though the presence of a bacterial film does not necessarily elicit the same response even among closely similar larval types (Crisp and Ryland, 1960; Ryland, 1974, 1976).

Mihm *et al.* (1981) show how different combinations of contrasting substrate with organic films, with or without bacteria present, effect the wettability of these surfaces and the subsequent variable response of larvae to them. This physical characteristic of surfaces, their wettability, even of clean surfaces free of contaminating films, also affects adherence of marine larvae (Eiben, 1976; Brewer, 1979; Mihm *et al.*, 1981). This demonstration of the interaction among larvae, water molecules, and surface characteristics clarifies otherwise inexplicable results when larvae are presented different material in the laboratory, and adds another dimension to be considered in the analysis of larval distribution in the field. The specific processes of attachment on suitable substrate to which motile larvae are compelled by their behavioral reflexes involve subtle mechanisms.

The planula of *Cyanea* shows behavioral responses bringing it into appropriate contact with substrate interpreted to increase the likelihood for survival of the polyp stage into which it develops following its initial encystment (Brewer, 1976a, b). The planula is a stereogastrula (Mergner, 1971; Campbell, 1974) possessing a slightly differentiated ciliated ectoderm surrounding a simple endoderm (*e.g.*, Widersten, 1978; Martin and Chia, 1982). It lacks complex sensory organs characteristic of the larvae of higher Metazoa, yet behaves with apparent equal effectiveness in selecting suitable substrate upon which to settle (Brewer, 1976a). The interaction with a surface and its immediate environment shown by planulae also parallels this response shown by the larvae of higher forms (Chia and Bickell, 1978), contributing both to a clearer interpretation of laboratory results and to a more complete understanding of their natural distribution in the field.

Planulae of *Cyanea* are exposed to different materials in various combinations to determine their behavior in contact with, and their attachment to, clean surfaces in the laboratory. Glass and plastic coverslips are the substrate used to ascertain the relationship between behavior and attachment of planulae, the effect that substrate orientation and texture has on their attachment, and the temporal sequence during which choice of these substrates is made. The frequency of attachment of planulae to these and to other inorganic substrates exhibiting a wide range of wettability is determined and compared to the wettability of natural substrate (shell) on which the attached stages of *Cyanea* frequently occur in the field.

MATERIALS AND METHODS

The experiments were done at $19 \pm 1.6^\circ\text{C}$ using filtered natural sea water (28‰) collected from the site from which the medusae bearing planulae were collected, and were exposed to fluorescent illumination approximating a diel cycle except during observations requiring twenty-four hours to complete. The planulae, borne on the oral folds of medusae, were obtained the same day as the experiments were set up. The coverslips (Arthur H. Thomas Co., Pennsylvania) of plastic and glass used as artificial substrate were supported about 3 mm above the bottom of their experimental containers by glass beads fastened to their corners with silicone aquarium cement, and were soaked in sea water for at least twenty-four hours, after which time they were thoroughly rinsed before planulae were exposed to them. Roughened substrate was prepared by rubbing the surface of plastic coverslips with sandpaper (100 grit).

Control observations were made, prior to carrying out the experiments described below, to determine the relative frequency of encounters by planulae with plastic or glass coverslips. Planulae were introduced into a 10 cm diameter bowl containing three coverslips of each type. The number of planulae encountering each coverslip

during a one minute observation period within a microscope field at 25 \times magnification (8.5 mm diameter) was recorded seven times at two hour intervals from between two to ten hours after their introduction ($n = 21$ for each coverslip type).

The behavior of planulae after contact is made with a substrate, compared both with respect to upper and under surfaces as well as to coverslip material, was recorded for 20 planulae (10 upper surface; 10 under surface) observed for three minutes on each substrate, and for 10 planulae on the under surface of each of 10 coverslips (5 plastic; 5 glass) where their behavior upon contact was noted and timed beginning eight hours after their introduction into the bowl ($n = 50$ for each coverslip type).

The attachment of planulae to plastic coverslips held at two different angles were compared for eight horizontally oriented coverslips and for eight vertically oriented coverslips; four coverslips at each orientation were roughened on both sides. The vertical coverslips, held at 90 $^\circ$ by paired glass beads fastened on opposite sides of each of the two lower corners, had their lower edge in contact with the dish bottom so that planulae could not swim beneath them. The four treatments were arranged in a Latin square in an 18 cm diameter bowl. The planulae were allowed to attach (36 hours) and the numbers on the upper and under surfaces (horizontal) and on either side (vertical) were counted.

The temporal sequence of attachment by planulae to plastic and to glass substrates, and to other surfaces in a 10 cm diameter bowl was determined by counting planulae attaching to three plastic and to three glass coverslips (upper plus under surfaces) at hourly intervals for twenty-four hours (except for hours 9, 16, and 18). The time that planulae began to attach to the hyponeuston, to the dish bottom beneath the coverslips, and to the upper surface of coverslips was also noted.

The relationship between attachment and the wettability of surfaces was found by determining the contact angle (measured with a goniometer eyepiece) formed by a 0.03 ml drop of distilled water on surfaces to which planulae were exposed; the contact angle measured is the same as that used by Eiben (1976) and Baier *et al.* (1968) based upon the description of Young (1805), except where otherwise noted, below. Five measurements at 20 \times magnification were made for each substrate between ten and ninety seconds after the drop was deposited; the temperature was 21 $^\circ$ C and the relative humidity, 75%. Four treatments (coverslips of glass and of plastic, and each of these materials coated with siliclad) were arranged in a 22 cm \times 34 cm glass dish as a modified 8 \times 8 Latin square giving 16 replicates for each treatment, and three treatments (glass coverslips polished with commercial lens cleaner, opal, and mica) each with 4 replicates were symmetrically arranged within this pattern. The total number of planulae attaching to each of these substrates was counted sixty hours after they were placed in the dish. The diameter of 50 attached planulae (planulocysts) on each coverslip (except those treated with lens cleaner) was measured to the nearest 0.5 graticule unit (1 graticule unit = 18.2 μ m) at 50 \times magnification using reflected illumination. These measurements were made on "blind samples," coded by an assistant, and in haphazard order relative to the treatments they may have represented. The contact angle formed on roughened coverslips and on several organic substrates, scallop and wave-worn pieces of other bivalve shells, was also determined. The scallop shells were obtained by dredging and were divided into three categories: fresh (removed from live individuals), non-eroded (lightly filmed and bearing newly settled barnacles), and eroded (lightly filmed exhibiting a bronzy iridescence and bearing planulocysts). Ten measurements of contact angles made by an air bubble on the inner surface of submerged scallop shells in each category were made (after the method of Mihm *et al.*, 1981) the same day they were collected.

One-way ANOVA is used to analyze these data; variances among treatments are homogeneous. The row and column effects for the Latin squares are non-significant ($0.25 < P < 0.50$ and $0.10 < P < 0.25$, respectively).

RESULTS

The planulae show no difference ($F_{1,90} = 0.15$; $0.50 < P < 0.75$) in their average number of encounters with either plastic (18.0 ± 16.2) or glass coverslips (16.1 ± 15.5) during the control observations. This ratio of contact between plastic and glass (1.1) does not change among the four time intervals during the eight hour observation period (0.8, 1.6, 1.1, 1.3). The probability of encountering these two substrates when they are simultaneously available to planulae is the same, and the differences in attachment on them in the experiments, below, cannot be attributed to different relative availability of these surfaces to planulae.

The response of planulae when encountering glass or plastic is shown in Figure 1. First, planulae on both materials show similar behavior with respect to coverslip surface: they appear to glide on the upper surface (just as they do on the bottom of their container) slowly rotating about their long axis in a clockwise direction when viewed from their aboral (anterior) pole; but on the under surface they become vertically oriented at a particular spot with their aboral end against it where they continue to rotate clockwise. Change of location is preceded by a brief quickening of rotation and a rapid lateral movement of 1–2 mm while still vertically oriented, followed by a loss of contact and swimming in an arc-shaped trajectory as shown in Figure 1, prior to resuming a position normal to the surface upon renewal of contact. Second, differences in the duration of rotation (seconds; 0.95 confidence limits) by the vertically oriented planula while against the under surface on glass (8.4; 6.6, 10.6) and plastic (14.0; 10.4, 18.9) is significant ($F_{1,99} = 7.35$; $0.005 < P < 0.01$). This difference is reflected in the number of planulae attaching to these two materials: after twenty-four hours, 30 individuals (7.9%) attach to glass, while 351 (92.1%) attach to plastic. On the under surface, planulae assume an orientation perpendicular to the substrate, and remain in this position longer on plastic than on glass; this results in a greater proportion of planulae attaching to plastic.

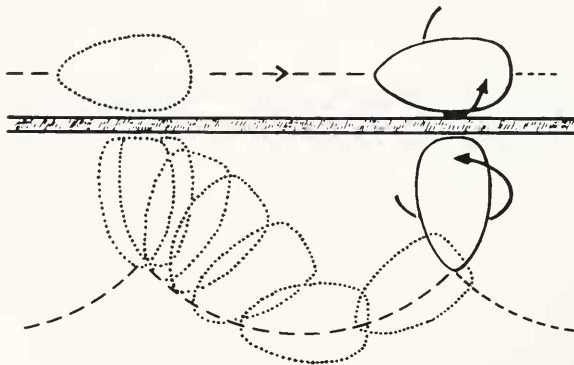


FIGURE 1. The behavior of planulae of *Cyanea* in contact with upper (top of figure) and under (bottom of figure) surfaces. The blunt end (aboral pole) is anteriormost during locomotion. Planulae on the upper surface glide with their long axis parallel to it (dashed arrow), rotating in the direction shown by the solid arrow. The direction of rotation is the same for planulae against the under surface, but they orient perpendicular to it for variable periods of time depending upon the quality of the surface; between these stationary periods of rotation, they move as shown by the dotted outlines along a path described by the curved dashed line.

Aboral contact normal to a substrate is not the sole stimulus leading to attachment. Planulae gliding along a container bottom, or swimming horizontally, collide with the surface of vertically oriented plastic coverslips with their aboral end, but few (348) attach there relative to horizontally held coverslips (5551) ($F_{1,12} = 41.22$; $P < 0.001$). The planulae which attach to the vertical coverslips are equally distributed on either side (165:183), but nearly ten times as many planulae attach to the under surface of horizontal coverslips (5021:530). Though more planulae attach to roughened (920) than to smooth (468) coverslips, there is no difference between them: for vertical coverslips ($F_{1,12} = 0.08$; $P > 0.75$), and for horizontal coverslips ($F_{1,12} = 1.64$; $0.10 < P < 0.25$). Planulae attach to a suitable substrate more frequently if that surface is properly oriented, regardless of its texture (see Discussion).

The temporal sequence of planular attachment to coverslips of plastic or glass is shown in Figure 2. Planulae attach to plastic (at 11.2 hours) 8.4 hours sooner than they attach to glass (at 19.6 hours). Attachment to glass is more variable than that for plastic (C.V. = 66.1% and 20.9%, respectively) and is not normally distributed; the solid curve for attachment to glass (fit by eye) is made parallel to the curve for attachment to plastic. However, even without statistical comparison the difference between attachment to glass and to plastic is clearcut. The actual exponential curve for attachment to glass coverslips (dashed curve, Fig. 2) occurs during the attachment of planulae to the hyponeuston beginning at seventeen hours, to the dish bottom at nineteen hours, and to the upper surface of coverslips at twenty-one hours. Planulae attach to plastic surfaces before they do to those of glass, but during their settlement on glass coverslips, they attach to other surfaces in their experimental container.

Three other comparisons between planular response to plastic and to glass can be made from this experiment of twenty-four hours duration. First, as above, the

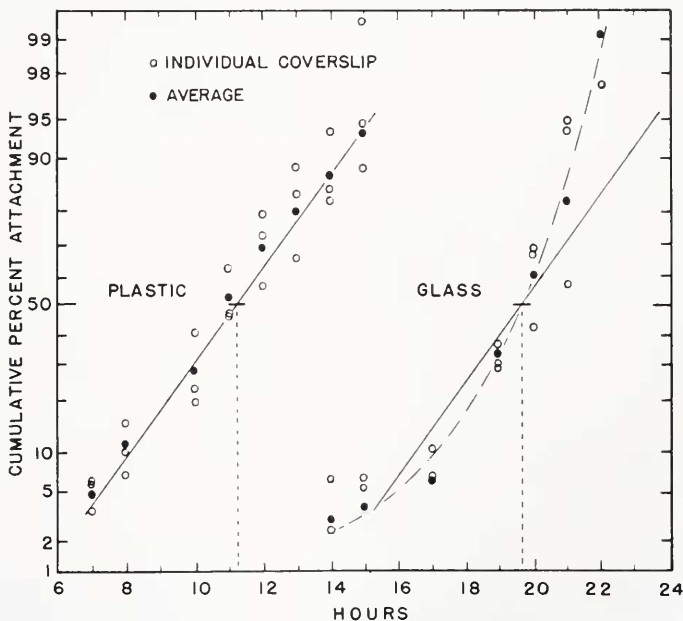


FIGURE 2. The temporal pattern of attachment of planulae of *Cyanea* to plastic and to glass coverslips. Where less than three individual coverslips are shown for an observation period, it (they) equal either 0% or 100%.

number of planulae attaching to plastic (2395) exceeds that for glass (84). Second, only 65 (2.7%) planulae attach to the upper surface of plastic coverslips (also, as above), but the attached planulae are evenly distributed between under and upper surfaces on glass coverslips (40 and 44, respectively). Third, equal numbers of planulae attach to the dish bottom beneath the plastic and glass coverslips (139 and 134, respectively) showing again (see above), but indirectly, that both coverslip types are equally accessible to the planulae.

The contact angle, or surface wettability, affects planular attachment (Fig. 3). Planulae vary in their attachment to surfaces which exhibit a contact angle less than 19° (lens cleaner-coated coverslips, $17.5 \pm 5.1^\circ$; opal, $17.3 \pm 1.9^\circ$; and mica, $18.9 \pm 1.8^\circ$), and at angles greater than this, but including the data for opal for the lowest point, their relative attachment to the substrates available to them directly parallels the size of the angle ($\bar{Y} = 52.4 + 2.7X$; $0.005 < P < 0.01$). The diameter of the planulocysts formed by the attaching planulae is also correlated with contact angle: the cysts formed on glass ($230.3 \mu\text{m}$) are smaller ($F_{1,196} = 6.62$; $P \approx 0.01$) than are those formed on plastic or either of the siliclad-treated coverslips ($300.0 \mu\text{m}$; $299.0 \mu\text{m}$; $295.9 \mu\text{m}$, respectively); there is no difference in size among these latter planulocysts ($F_{2,196} = 0.68$; $0.25 < P < 0.50$). The contact angle for roughened plastic coverslips ($80.1 \pm 2.2^\circ$) on which more planulae attach (see above) is larger than that of their smooth counterparts ($67.6 \pm 3.5^\circ$) and is close to the contact angle determined for siliclad-coated coverslips ($81.6 \pm 3.6^\circ$) on which most planulae settle (Fig. 3). On natural substrates to which planulae are exposed in the field, the contact angle on fresh scallop shell ($18.8 \pm 3.0^\circ$) approximates that for opal, mica, and lens cleaner-coated coverslips upon which settlement is relatively low and variable; wave-worn

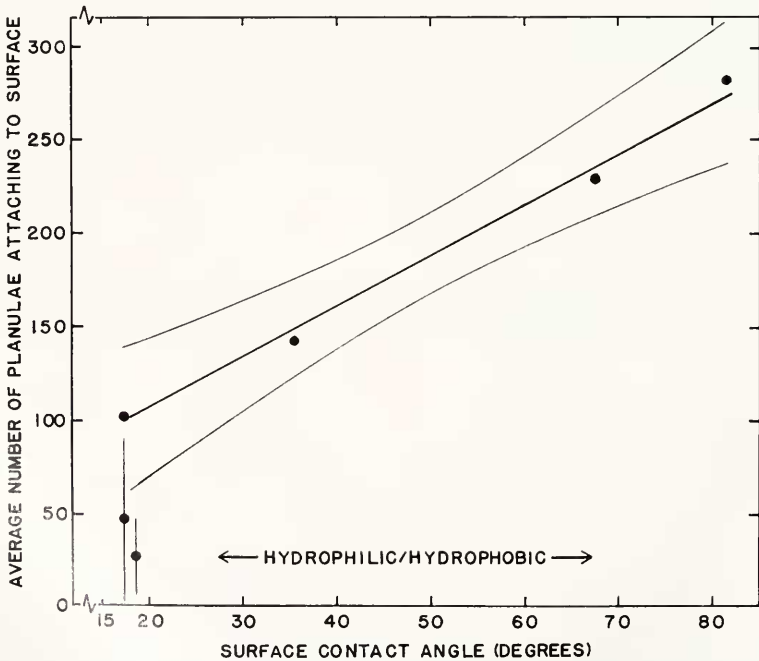


FIGURE 3. The average attachment (and 0.95 confidence limits) of planulae of *Cyanea* exposed sixty hours to surfaces with different contact angles. See text for identification of surface materials.

shells ($30.9 \pm 7.5^\circ$) and non-eroded shells ($35.9 \pm 5.1^\circ$) show larger contact angles similar to that for glass ($35.9 \pm 3.7^\circ$); and the largest contact angle for organic substrate is found on eroded scallop shells bearing planulocysts ($52.4 \pm 2.6^\circ$). *Cyanea* planulae attach most readily to inorganic surfaces which are relatively hydrophobic, and this is apparently also true for organic substrates which they normally encounter in the field (See Discussion).

DISCUSSION

Cyanea planulae are influenced by the relative hydrophobicity of hard surfaces, not only in their actual attachment but also in the correlates of attachment (duration of inspection, time required to attach, size of encysted planulae). The more non-wettable or hydrophobic the surface, indicated by large contact angles, (1) the longer the period of inspection, (2) the shorter the time to attach, (3) the higher the frequency of attachment, and (4) the larger the encysted planulae. The interpretation of the relationships among the process of inspection, the time to attach, and actual attachment is straightforward; the size of the cyst, on the other hand, is a manifestation of surface properties associated with adherence once contact is achieved. The cyst produced by planulae upon attachment is a measure of the size attained when their attachment is complete, and may be considered to be a multicellular analogue to metazoan cells in tissue culture. Such cells exhibit greater spreading on surfaces to which they adhere (e.g., Weiss, 1961; Weiss and Blumenson, 1967; Baier, 1980), and planulocysts are larger in diameter on surfaces to which adherence is most readily obtained (hydrophobic surfaces). Planular response to clean, smooth surfaces in the laboratory demonstrate physical constraints imposed upon their attachment to them; this has been shown for other marine larvae.

The response of planulae to surfaces exhibiting different wettability is not unique. The results obtained by Eiben (1976) and Mihm *et al.* (1981), both of whom used larvae of Bryozoa, show this phenomenon. Eiben (1976; see also Müller *et al.*, 1976) describes a predictive model to account for his observations using the larvae of *Bowerbankia* exposed to surfaces of different wettability. This model is based on a well known principle of thermodynamics, that in any system surface free energy will tend to a minimum (e.g., Steinberg, 1964). Larvae cannot settle upon surfaces with high wettability (small contact angles), for this combination of larval surface and substrate does not lead to the greatest reduction in surface free energy; the larvae cannot overcome the bonding strength of the particular configuration of water molecules in contact with an hydrophilic surface (e.g., Tanford, 1973). The largest contact angle at which larvae cannot attach Eiben (1976) calls the "critical angle;" the critical angle for *Bowerbankia* is 17° , and for *Bugula* (Mihm *et al.*, 1981) it is at least as large as 45° . No unambiguous critical angle is demonstrated for planulae over the range of contact angles examined, but the variation in their response to contact angles between 17° and 19° if not due to other causes might indicate that they could not attach to surfaces exhibiting a slightly greater wettability. While the details differ, the planulae of *Cyanea* show a limited capacity for attachment to highly wettable surfaces in general agreement with the model proposed by Eiben (1976).

Another prediction of Eiben's (1976) model is that larval attachment will be uniformly high on surfaces with contact angles exceeding the critical angle. He shows this for *Bowerbankia*, and the data for *Bugula* (Mihm *et al.*, 1981) also support such an "all-or-nothing" response. However, over the range of contact angles permitting attachment, the planulae of *Cyanea* show a response proportional to the hydrophobicity of the surface. This pattern for planulae is probably due to differences in experimental

procedure rather than reflecting disagreement with the model of Eiben (1976) and the results of Mihm *et al.* (1981) for Bryozoa. Their data represents the proportion of larvae attaching out of the total number of larvae exposed for short duration, to a single substrate; mine, the relative numbers of planulae selecting a particular substrate when presented with a choice among several during prolonged exposure. The different time element is dictated by larval characteristics: the short-lived, synchronously settling bryozoan larva compared to the longer-lived, asynchronously settling planulae. However, the correlation in the numbers of planulae of *Cyanea* attaching to surfaces of different wettability emphasizes the relationship between their attachment and this surface property of solids.

The attachment of the larvae of *Bowerbankia* to non-wettable surfaces is described by Eiben (1976) as an exergonic process. This does not completely explain the interaction of planulae with hydrophobic surfaces, for it leads to the expectation that both their behavior and frequency of attachment to upper and to under surfaces of plastic coverslips, or to vertically held plastic coverslips, should be more alike than is observed. The temporal sequence of planular attachment shows planulae finally settling (literally?), and in an atypical location, on the upper surface of coverslips (and on the dish bottom) only when their metabolic reserves become largely depleted (?) relatively late during their free-swimming phase. Planulae do not indiscriminately respond passively to suitable substrate, but only to this substrate when it is horizontally oriented and they approach it from below.

Substrates in the field exhibit both different textures and variable degrees of fouling; both roughness and fouling alters the wettability of the original surface. Though the accurate measurement of contact angle on rough surfaces is difficult (Baier *et al.*, 1968), empirical results generally agree with theory: roughening the surface of a solid lowers its surface energy (Johnson and Dettre, 1964). The change in wettability that altering the texture of coverslips effects, and the attachment of planulae of *Cyanea* and of other Scyphozoa to them, is shown in Table I. Planulae of *Cyanea*, *Aurelia*, and *Chrysaora* attach to a greater extent on the rough, more hydrophobic surface. While only Brewer's (1976a) data shows significantly more planulae attaching to rough surfaces, these consistent results suggest that planular attachment to roughened substrate might represent their response to the change in wettability caused by surface alteration rather than to texture, *per se*. This may be why scyphozoan larvae are not found more frequently on rough substrate in the field (Cargo, 1979; Brewer, in prep.) for even a smooth surface with a large contact angle, for whatever cause, would provide an equally suitable surface upon which to settle.

The smooth inner surface of bivalve shells is a common site for the attachment of planulae of *Cyanea* in the field (Brewer, in prep.), but clean shells exhibit small contact angles equivalent to those of inorganic surfaces relatively unfavorable for planular settlement; this may explain why repeated attempts to obtain planular attachment on such shells in the laboratory have met with only sporadic success (Brewer, unpubl.). Mihm *et al.* (1981) show this for clean oyster shell finding it a poor substrate for the attachment of *Bugula*. However, clean surfaces quickly become fouled with an organic film followed by bacterial colonization when immersed in sea water (Zobell, 1943; Baier, 1970, 1980; Fletcher, 1979). The composition of adherends may differ qualitatively and/or quantitatively depending upon the wettability of the initial surface (Baier, 1980), with the nature of the organic film influencing bacterial attachment, and the two together, that of larvae: oyster shell allowed to become filmed and colonized with bacteria exhibit low wettability and a correspondingly high attachment to it by *Bugula* (Mihm *et al.*, 1981). The differences in the wettability of scallop shells becoming increasingly more hydrophobic with length of immersion is indirect evidence

TABLE I

The relative number of planulae of Scyphozoa attaching to rough and to smooth coverslips

Species (reference)	Number of planulae on coverslips (contact angle)		Ratio Rough/smooth
	Rough (80.1°)	Smooth (67.6°)	
<i>Cyanea</i> (this study)	920	468	1.97
<i>Cyanea</i> (Brewer, 1976a)	18603	11537	1.61
<i>Cyanea</i> (Brewer, unpubl.)	852	469	1.82
<i>Aurelia</i> (Brewer, 1978)	237	191	1.24
<i>Chrysaora</i> (Cargo, 1979)	98136	71010	1.38

that surface changes (through erosion) and/or the presence of films, altering their wettability, is necessary for the attachment of the planula of *Cyanea* in the field. Planulae have never been found attached to fresh shells, and the attached stages of *Cyanea* are most commonly encountered on older, eroded ones showing a bronzy iridescence on their inner surface (pers. obs.).

The correlation between larval settlement and surface wettability describes permissible conditions for their attachment, but not the mechanism of adhesion nor the stimulus for subsequent metamorphosis. The close contact of small and weakly powered larvae allowed by nonwetable substrates permits the occurrence of an effective physical stimulus, shearing forces exerted on cilia, initiating the final attachment of *Bowerbankia* (Eiben, 1976) and of *Hydractinia* (Müller *et al.*, 1976). Perhaps such proximity brought about by appropriately wettable surfaces is a requisite for other larvae for which chemical mechanisms are implicated: for example, the enzyme-like reaction between surface lectins of larvae and the polysaccharides produced by bacterial films proposed by Kirchman *et al.* (1982b) for the larval attachment of Bryozoa. The mechanisms, physical, chemical, or whether a combination of both, initiating the transformation of the planulae of *Cyanea* into a lenticular-shaped disc when completely attached (see Brewer, 1976a; Fig. 1) are not known.

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